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# Is resveratrol a potential substitute for leuprolide acetate in experimental endometriosis?

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ABSTRACT

*Objective:* Resveratrol, a phytoalexin polyphenol, has anti-angiogenic, antioxidant, anti-inflammatory properties. We aimed to compare the anti-inflammatory and anti-angiogenic effects of resveratrol and leuprolide acetate (LA) in an experimental endometriosis model.

*Study design:* A prospective experimental study was conducted in a University Surgical Research Center. Thirty-three non-pregnant female Sprague-Dawley rats, in which experimental model of endometriosis were surgically induced were randomly divided into four groups. Group 1 was administered 30 mg/kg resveratrol i.m. for 14 days, group 2 was given 1 mg/kg s.c. single dose LA, group 3 was administered both resveratrol and LA, and group 4 had no medication. After two weeks medication rats were sacrificed and size, histopathology and immunreactivity to matrix metalloproteinase (mmp)2, mmp9, vascular endothelial growth factor (VEGF) of the endometriotic implants were evaluated. Plasma and peritoneal fluid levels of interleukin (IL)-6, IL-8, and tumor necrosis factor-α (TNF-α) were analyzed.

*Results:* The endometriotic implant volumes, histopathological grade and immunreactivity to mmp2, mmp9 and VEGF were significantly reduced ($p < 0.001$), and plasma and peritoneal fluid levels of IL-6, IL-8 and TNF-α were significantly decreased in group 1 and group 2 in comparison to group 3 and group 4 ($p < 0.001$).

*Conclusion:* Resveratrol alone is a potential agent for the treatment of endometriosis and may be an alternative to LA. In contrast, the combination of LA and resveratrol decreased the anti-inflammatory and anti-angiogenic effects of each agent. Since resveratrol is widely used as an alternative therapy for a variety of conditions, it can undermine the effectiveness of LA. Therefore, caution should be exercised when used in combination with other agents.



## Introduction

Endometriosis is an estrogen-dependent disease characterized by the presence of endometrial glands and stroma outside of the uterine cavity [1]. Endometriosis is a common disease of reproductive-age women and associated with chronic pelvic pain, dysmenorrhea, dyspareunia, and infertility [2].

Although endometriosis is one the most investigated disorders of gynecology, it's pathogenesis remains unclear and has been based on two theories: retrograde menstruation and coelomic metaplasia [3,4]. Further investigations have demonstrated a role for immune dysregulation in the microenvironment of the peritoneal fluid for the development of endometriosis, including elevated levels of cytokines and activated macrophages with reduced phagocytic activity in the peritoneal fluid [5]. The development and maintenance of the endometrial implants depend on the implantation, differentiation, invasion of endometrial cells, and their neovascularization. Medical treatments have been aimed at maintaining a hypo-estrogenic environment by suppressing the hypothalamic–pituitary axis with gonadotropin releasing hormone (GnRH) analogues, but the side effects of these therapies limit their long-term usage.

Resveratrol, a phytoalexin polyphenol, is a compound found in red wine, grapes, and berries. The anti-angiogenic, antioxidant, anti-inflammatory properties of resveratrol have been well established. It has been found to be beneficial for cardiovascular

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diseases, cancer, type-2 diabetes mellitus, and neurodegenerative diseases [6,7]. The aim of this study was to evaluate the therapeutic effect of resveratrol and to compare this effect with a GnRH analogue, leuprolide acetate (LA). To our knowledge, no studies comparing the effects of resveratrol with LA on surgically-induced endometriosis in a rat model have been conducted.

## Materials and methods

The Black Sea Technical University Committee on the Use and Care of Animals approved the experimental procedure and all investigations were performed in compliance with international guidelines on the ethical use of animals. The study was performed at the Surgical Research Center of Black Sea Technical University, Trabzon, Turkey.

Forty mature, non-pregnant female Sprague-Dawley rats weighting 200–250 g were used for the induction of the experimental endometriosis model. The guidelines for care and use of animals that were approved by the institutional review board were followed.

Before the surgical induction of endometriosis, the rats underwent daily vaginal lavages between 8:00 a.m. and 10:00 a.m. to detect the estrus cycle. Vaginal secretions were examined under a light microscope to identify the estrus cycle by the dominancy of the anucleate cornified cells [8].

### *Surgical procedure*

*Step 1: Establishment of the endometriosis model*

For anesthetizing the rats, ketamine hydrochloric acid (Ketalar; Eczacibasi Warner-Lambert Ilac Sanayi, Levent, Istanbul, Turkey) 50 mg/kg and xylazine hydrochloric acid (Rompun, Bayer Sisli, Istanbul, Turkey) 7 mg/kg were administered intraperitoneally. Using sterile surgical techniques, a 4–5 cm vertical midline incision was made to expose the bilateral uterine horns. The experimental endometrial model was induced by transplanting autologous fragments of endometrial tissue onto the inner surface of the abdominal wall as described by Vernon and Wilson [9] with the modifications of Lebovic et al. [10]. A 1 cm segment was removed from the right uterine horn after ligation with a polypropylene 4–0 suture. The excised fragment was immersed in sterile phosphate-buffered saline (PBS) solution and opened longitudinally from the anti-mesenteric side. The endometrial tissue was trimmed to 5 × 5 mm without removing any myometrial tissue. The fragment was transplanted onto the inner surface of the right abdominal wall and secured with non-absorbable polypropylene 4–0 suture. Before the enclosure of the abdominal wall, 2 mL of saline was put into the abdominal cavity to prevent drying of the serosal surfaces and to minimize adhesions. An uninterrupted 3–0 polyglactin 910 suture was used for the closure of the peritoneum and fascia and a simple interrupted 2–0 silk was used for the skin. After the surgery, all rats were caged individually and their body weight and wound healing were observed without any medication.

*Step 2: Measurement of endometrial implants*

At the second exploratory laparotomy, ectopic endometrial tissues were identified and three dimensional measurements were performed (length x width x height) using a caliper. The prolate ellipsoid formula was used for calculation of the spherical volume of each ectopic uterine tissue: $V\,(\mathrm{mm}^3) = 0.52 \times \text{length} \times \text{width} \times \text{height}$. All implants were photographed and their sizes and volumes were recorded (Fig. 1a). After the second laparotomy, two rats died because of anesthesia complications, and no implant was detected in five of the remaining animals. The remaining 33 rats were randomly divided into four groups. Group 1 was given 30 mg/kg resveratrol i.m. (R5010, Sigma-Aldrich TM Co, Saint Louis, MO, USA) for 14 days, group 2 was given 1 mg/kg s.c. single dose LA, group 3 was administered both resveratrol and LA, and group 4 had no medication. All rats were observed for 14 days as it was shown that LA begins to exert its effect earlier than two weeks [11].

*Step 3: Evaluation of the outcomes*

At the end of 14 days, the rats were euthanized with ketamine, and a laparotomy was performed. Before excising the endometrial implants, peritoneal lavage with 2 mL saline was performed to assess the inflammatory markers in the peritoneal fluid. The sizes of the implants (Fig. 1b) were measured, and their volumes were calculated. For the histopathological examination, the implants were fixed in 10% formalin. A blood sample was obtained by cardiac puncture using 5 mL syringe for the detection of the cytokine levels of the plasma.

### *Biochemical analysis*

Blood and peritoneal fluid samples were centrifuged at 3000 rpm for 10 min at room temperature and stored at −20 °C before the biochemical assay.

Serum and peritoneal fluid levels of rat TNF-α and IL-6 were quantified by enzyme-linked immunosorbent assay (ELISA) using commercially available matched antibodies (for TNF-α and IL-6:

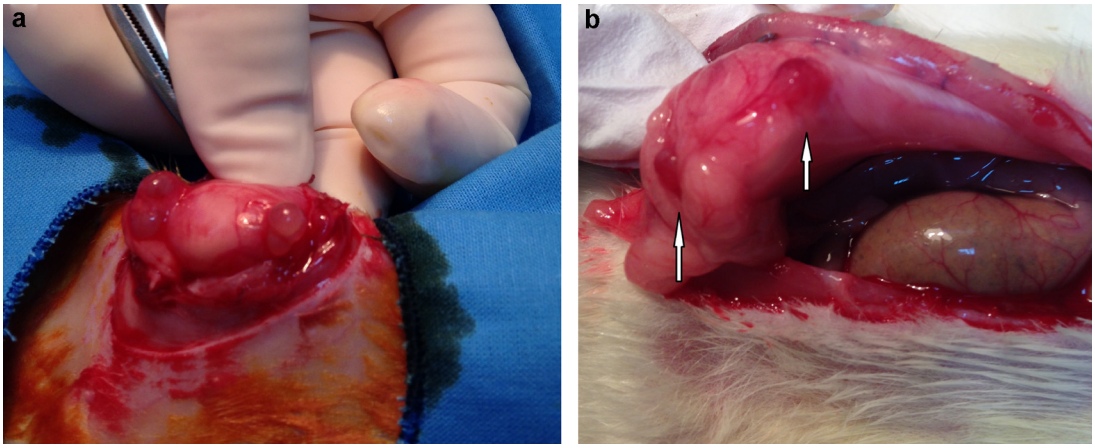


**Fig. 1.** The macroscopic appearance of endometriotic implants: two pre-treatment hypertrophic cystic implants in the abdominal wall (a), and the same implants after therapy (b). Arrows denote atrophic implants.